

Quorum sensing: How bacteria can coordinate activity and synchronize their response to external signals?

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Received 22 June 2012; Revised 18 July 2012; Accepted 18 July 2012

DOI: 10.1002/pro.2132

Published online 23 July 2012 proteinscience.org

Abstract: Quorum sensing is used by a large variety of bacteria to regulate gene expression in a cell-density-dependent manner. Bacteria can synchronize population behavior using small molecules called autoinducers that are produced by cognate synthases and recognized by specific receptors. Quorum sensing plays critical roles in regulating diverse cellular functions in bacteria, including bioluminescence, virulence gene expression, biofilm formation, and antibiotic resistance. The best-studied autoinducers are acyl homoserine lactone (AHL) molecules, which are the primary quorum sensing signals used by Gram-negative bacteria. In this review we focus on the AHL-dependent quorum sensing system and highlight recent progress on structural and mechanistic studies of AHL synthases and the corresponding receptors. Crystal structures of LuxI-type AHL synthases provide insights into acyl-substrate specificity, but the current knowledge is still greatly limited. Structural studies of AHL receptors have facilitated a more thorough understanding of signal perception and established the molecular framework for the development of quorum sensing inhibitors.

Keywords: quorum sensing; acyl homoserine lactone; synthase; bacterial signalling

Introduction

Numerous bacterial species correlate group activity in a cell-density-dependent manner using an inter-cellular signaling system known as quorum sensing (QS).^{1,2} This signaling system is contingent on the ability of such bacteria to constitutively synthesize small signal molecules intracellularly,³ which are then passively,⁴ or actively⁵ exchanged with the surrounding environment. Accumulation of the signal

molecules is thus commensurate with the increase in bacterial population, and when the population density exceeds a “quorate” threshold, the corresponding levels of signal can induce a synchronized response in gene expression throughout the population. These signal molecules, termed autoinducers,⁶ trigger the QS process by binding to a cognate receptors, which in turn regulates transcription of many genes that are involved in the cell-density-dependent behavior.¹

Autoinduction was first discovered in 1970 in the bioluminescent Gram-negative bacterium *Vibrio fischeri*,⁷ which led to the appreciation of a QS

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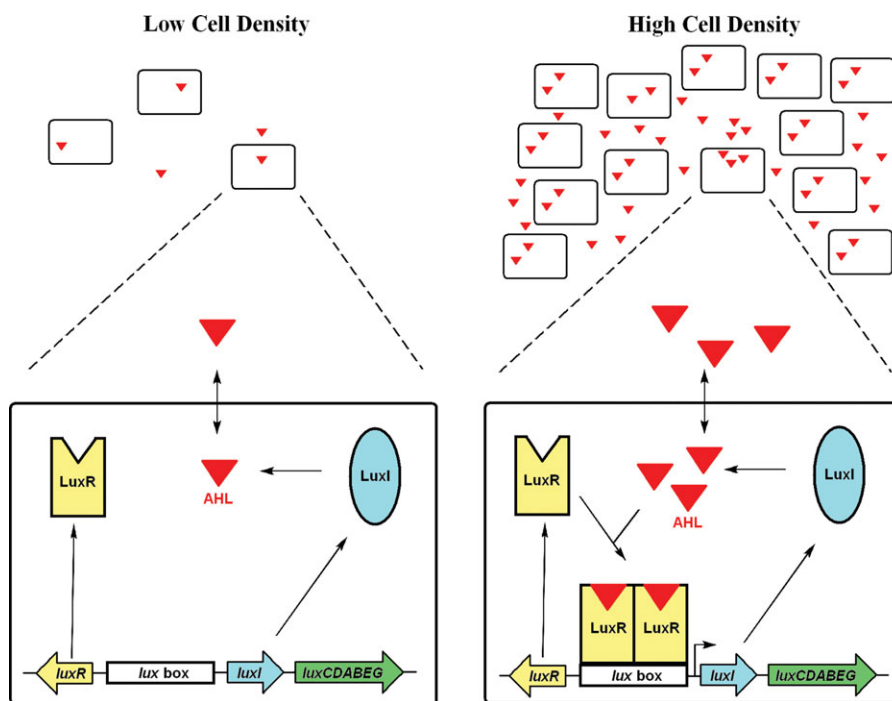


Figure 1. Acyl homoserine lactone (AHL)-dependent quorum sensing system as exemplified by LuxI/R system in *V. fischeri*.

system in this organism.^{1,6,8} *V. fischeri* is a symbiont of the Hawaiian bobtail squid *Euprymna scolopes* and lives in its light organ, whose rich nutrients allow fast proliferation of the bacteria.⁹ When the bacteria density is sufficiently high, genes involved in bioluminescence are expressed and light is produced to provide an antipredatory response by preventing the squid from casting a shadow under moonlight.^{10,11}

To date, QS has been described for many species and plays vital roles in diverse cellular functions of both Gram-negative and Gram-positive bacteria. In addition to regulating bioluminescence of *Vibrio harveyi*,¹² QS can also control root nodulation by nitrogen-fixing symbiont *Bradyrhizobium japonicum*.¹³ One of best characterized roles for QS is in regulating the behavior of bacterial pathogens, including virulence gene expression,^{14–16} biofilm formation,^{17,18} swarming,¹⁹ antibiotic production,²⁰ and antibiotic resistance.²¹ Pathogens can also use QS as a strategy to coordinate their interactions with the host. For example, the opportunistic human pathogen *Pseudomonas aeruginosa* relies on QS to evade the host immune response and develop antibiotic resistance.^{15,22–24} *P. aeruginosa* cells can coordinate to recognize an attack from human innate immune system and, in response, upregulate the expression of virulence determinants involved in the formation of protective biofilms.^{23–25}

The critical function of QS in pathogen infection has led to numerous efforts toward the development of novel antimicrobials that target the QS system.^{15,26–30} In contrast to traditional bacteriocidal or bacteriostatic antibiotics, disrupting QS does not

cause lethality but rather inhibits pathogen virulence.^{15,26,31,32} Thus, QS inhibitors have a potential advantage over other antibiotics that they may exert weaker selective pressure and thus are less likely to result in multidrug resistance.^{26,33}

The development of QS inhibitors has been facilitated by the increasing knowledge of the mechanisms of QS, including an understanding of autoinducer syntheses and corresponding receptors (see review³⁴). Major types of autoinducers include the acyl-homoserine lactones (AHL) in Gram-negative bacteria,³⁵ modified oligopeptides in Gram-positive bacteria,^{36,37} and a class of 4,5-dihydroxy-2,3-pentanedione-derived signal molecules termed autoinducer-2 (AI-2) in both Gram-negative and -positive bacteria.^{38–42} Several other autoinducers have also been reported, including 3OH palmitic acid methyl ester (3OH PAME),⁴³ cyclic dipeptides,⁴⁴ *Pseudomonas* quinolone signal (PQS),⁴⁵ diffusible signal factor (DSF),⁴⁶ and cholerae autoinducer-1 (CAI-1).^{47,48} The AHL-dependent QS system is studied the best so far and the focus of this review will be on the structural and mechanistic basis for signal production and perception in this system. Although there have been a few structural studies reported for AI-2 dependent^{40,49–54} and other QS systems,^{55–60} they will not be discussed here.

Acyl homoserine lactone (AHL)-dependent QS

AHL molecules are used as the primary QS molecules in Gram-negative bacteria.³⁵ They are produced by cognate AHL synthases and accumulate both in the cell and in the environment (Fig. 1). The

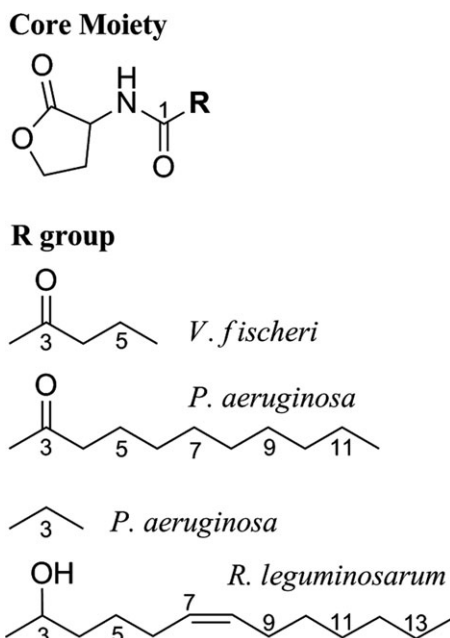


Figure 2. Structures of acyl-homoserine lactone molecules produced by different bacteria.

concentration of AHL molecules increases as the bacteria population grows. When the population density reaches the “quorum,” these AHL molecules exceed the threshold concentration and are recognized by specific receptors that belong to a large class of DNA-binding transcription factors named “R-proteins,” such as LuxR in *V. fischeri*^{6,61} and LasR in *P. aeruginosa*.^{62,63} The R-proteins, upon binding to the specific AHL molecules, directly regulate the transcription of target genes, by binding to^{1,62,63} or dissociating from,⁶⁴ corresponding promoters. For example, upon binding to its cognate signal AHL molecule, LuxR in *V. fischeri* binds to a short sequence termed *lux* box, and activate the transcription of the downstream operon, *luxICDABEG*, which contains the *luxI* gene that encodes the AHL synthase¹ (Fig. 1).

Diverse AHL signal molecules are produced by different species for specific intraspecific communica-

tion. These molecules all share a common homoserine lactone (HSL) ring, but vary in the length, backbone saturation, and side-chain substitutions (usually 3-oxo or 3-hydroxyl groups) of the fatty acyl chains (Fig. 2).⁶⁵ For example, *V. fischeri* produces 3-oxo-C6-HSL,⁷ *P. aeruginosa* produces both 3-oxo-C12-HSL⁶⁶ and unsubstituted C4-HSL,^{67,68} and *Rhizobium leguminosarum* produces 3-hydroxy-7-cis-C14-HSL.⁶⁹ Importantly, each of the AHL molecules is synthesized by a dedicated, cognate AHL synthase, and these enzymes do not show any promiscuity. For examples, the two signals produced by *P. aeruginosa*, 3-oxo-C12-HSL and C4-HSL, are synthesized by two different, independent synthases, LasI and RhII, respectively.⁷⁰

AHL Synthases

Three AHL synthase families have been identified so far, and these include the LuxI (see review³⁴), HdtS,^{71,72} and LuxM^{12,73,74} families. Among the three the LuxI family is the best studied as the LuxI protein in the *lux* operon in *V. fischeri* was the first AHL synthase to be identified⁶¹ (Fig. 1). LuxI homologs have been described in a large number of Gram-negative bacteria.^{35,75} Biochemical studies, both *in vitro*^{76–78} and *in vivo*,⁷⁹ demonstrate that enzymes in the LuxI family use *S*-adenosyl-methionine (SAM) and acyl-acyl-carrier-protein (acyl-ACP) as substrates to produce AHL molecule (Fig. 3). In contrast to members of the LuxI family, the HdtS and LuxM types of AHL synthases have been found in only a few bacterial species and less is known about the enzyme mechanism of these synthases. However, it appears that LuxM enzymes also use SAM as one of its two substrates and are capable of using either acyl-ACP or acyl-coenzyme A (acyl-CoA) as the other substrate.⁸⁰

Interestingly, a subfamily of LuxI homologs was recently classified based on their preference for acyl-CoA over acyl-ACP substrates. This subfamily includes RpaI from *Rhodopseudomonas palustris* CGA009,⁸¹ BraI from *Bradyrhizobium* BTAi1,⁸² and

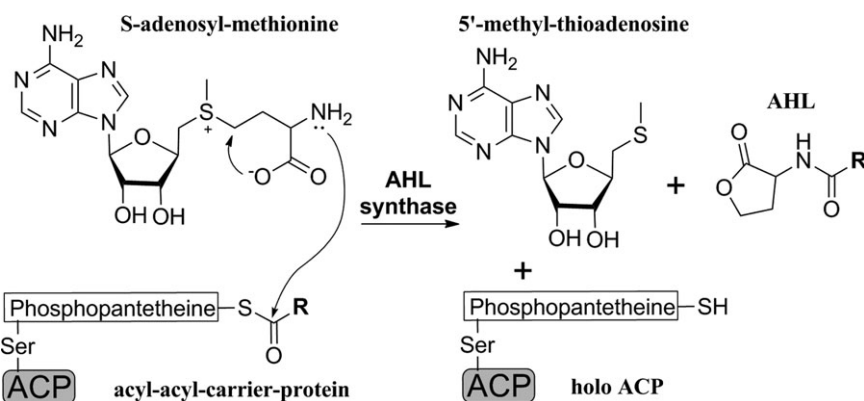


Figure 3. Reaction scheme of the synthesis of *N*-acyl-homoserine lactone catalyzed by AHL synthase.

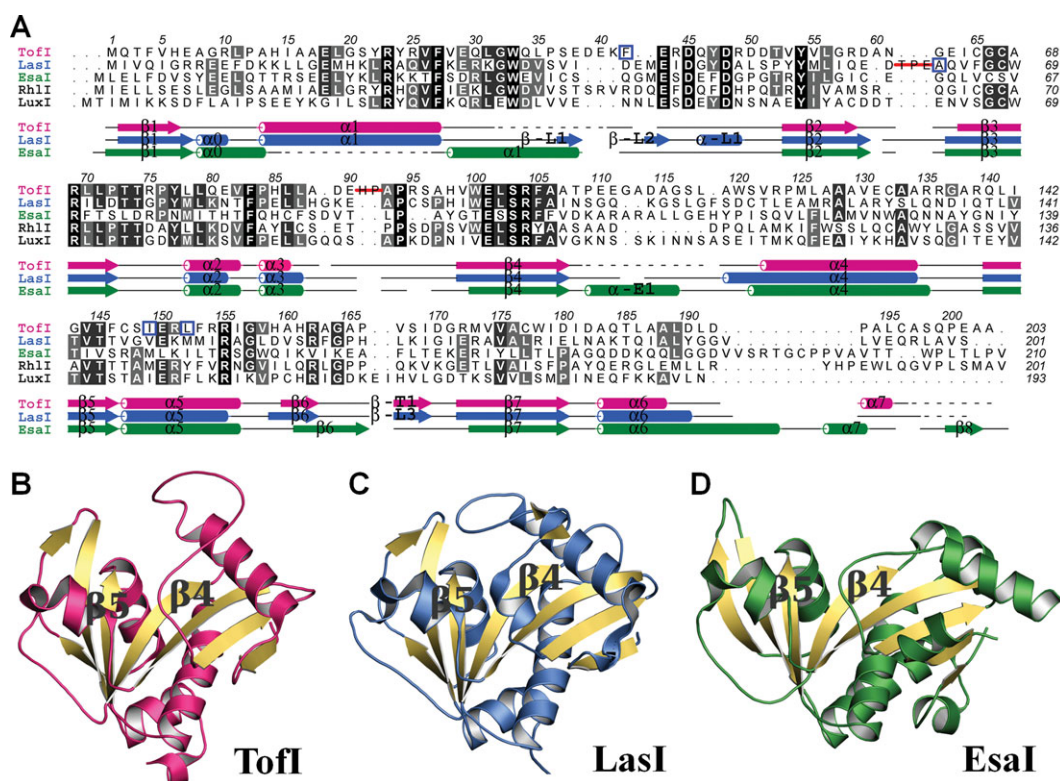


Figure 4. Sequences and structures of three AHL synthases. (A) Structure-based sequence alignment of the AHL synthases. Identical residues are shaded. Residues deleted or substituted in order to facilitate crystallization are indicated by red crosslines or blue frames. Secondary structures of TofI, LasI, and EsaI are shown below the sequences. (B–D) Three-dimensional structures of TofI, LasI, and EsaI. The stands $\beta 4$ and $\beta 5$ are labeled.

BjaI from *Bradyrhizobium japonicum* USDA110.⁸³ Although these enzymes are homologous to LuxI in primary sequence, they differ from canonical LuxI enzymes in two ways. First, their products have chemical structures that are distinct from typical AHL molecules, as RpaI, BraI, and BjaI produce p-coumaroyl-HSL, cinnamoyl-HSL, and an unusual branched-chain signal isovaleryl-HSL, respectively. Second, they are proposed to use CoA-linked rather than ACP-linked substrates. RpaI and BjaI have been shown to have substantial activity using p-coumaroyl-CoA⁸¹ and isovaleryl-CoA⁸³ as substrates, respectively.

Before the elucidation of the structures of LuxI enzymes, knowledge of the mechanisms of LuxI-type AHL synthases were largely derived from mutational analyses of LuxI from *V. fischeri*⁸⁴ and RhlI⁷⁰ from *P. aeruginosa*. These studies identified seven residues that are conserved in LuxI-family protein and are proposed to be involved in catalysis and SAM binding. All seven residues mapped to an N-terminal region between residues 24 and 104 (using RhlI numbering), including six charged residues (Arg24, Glu46, Asp48, Asp51, Arg71, and Arg104) and one neutral residue (Gly68) [Fig. 4(A)]. The C-terminal region of I-proteins is less conserved and is proposed to recognize the acyl-ACP substrate, which is variable for different AHL synthases.^{70,84}

Overall structure of LuxI-type AHL synthases

To date crystal structures of three LuxI-family proteins have been reported: EsaI from plant pathogen *Pantoea stewartii*,⁸⁵ LasI from *P. aeruginosa*,⁸⁶ and TofI from the plant pathogen *Brukholderia glumae*.⁸⁷ The products of these three enzymes are 3-oxo-C6-HSL, 3-oxo-C12-HSL, and C8-HSL, respectively. EsaI, LasI, and TofI are all around 200 residues in length and share less than 20% sequence identity, but are structurally similar, with an average RMSD of 2.3 Å over approximately 155 C α residues.⁸⁸ They all exhibit a single-domain structure with an α - β - α fold, with a highly twisted antiparallel β -sheet sandwiched between two groups of α -helices (Fig. 4). Further description of structural details and residue numbering will be based on TofI, unless otherwise stated.

The most conserved regions include seven strands $\beta 1$ – $\beta 7$ that constitute the central β -sheet, two short helices $\alpha 2$ and $\alpha 3$, and two long helices $\alpha 4$ and $\alpha 5$. A β -bulge between two invariant residues Ser103 and Arg104 in strand $\beta 4$ is conserved in all three AHL synthases and creates an apparent V-shaped cleft between bent strands $\beta 4$ and $\beta 5$, with the concave side facing a prominent pocket [Fig. 5(A)]. The pocket is enclosed mainly by strands $\beta 4$, $\beta 5$, and $\beta 7$, and helices $\alpha 4$ and $\alpha 5$, which are all well conserved structural elements.

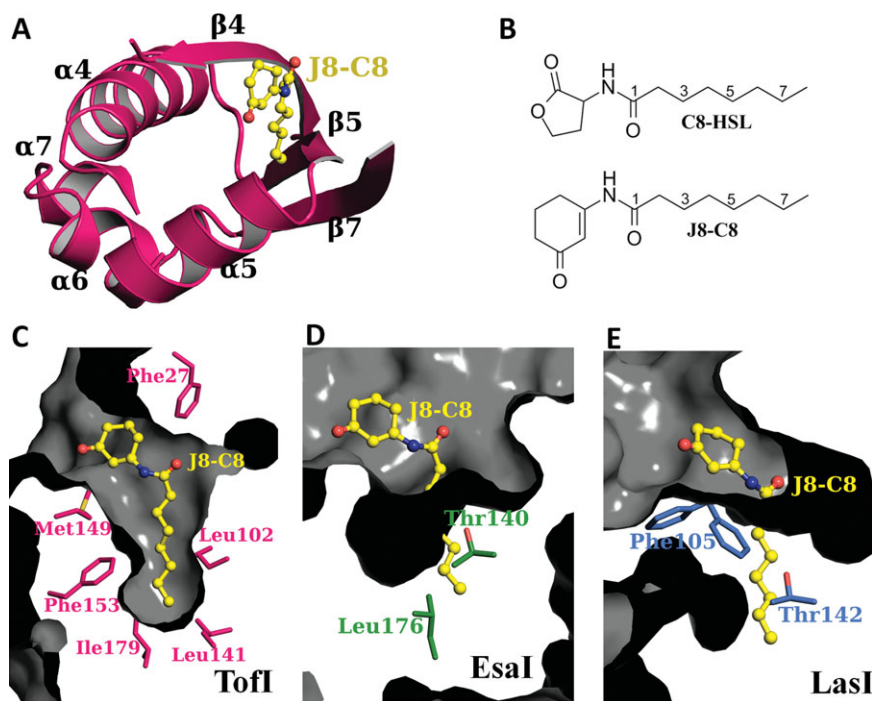


Figure 5. Acyl-chain binding in TofI. (A) The secondary structure elements that form the acyl-chain binding pocket. (B) Chemical structures of C8-HSL and J8-C8, the product and inhibitor, respectively, of TofI. (C) Surface representation of the acyl-binding tunnel in TofI. The hydrophobic residues that form the tunnel are shown. (D) Superimposition of the TofI ternary structure and the EsaI structure. Only J8-C8 is shown from the TofI structure. (E) Superimposition of the TofI ternary structure and the LasI structure. Only J8-C8 is shown from the TofI structure.

An acyl-chain binding tunnel identified in TofI

While EsaI and LasI have only been structurally characterized in their apo form, the more recently determined TofI structures report both the apo form, as well as that of the ternary complex with 5'-methylthioadenosine (MTA) and J8-C8, a synthetic analog of the product C8-HSL [Fig. 5(B)]. J8-C8 is shown to be a competitive inhibitor of TofI activity both *in vivo* and *in vitro*.⁸⁷ The ternary complex structure provides molecular insights into the probable binding mode between an AHL synthase and its substrates.

Within the TofI-MTA-J8-C8 ternary complex, the product analog inserts into a prominent tunnel enclosed in the pocket described above [Fig. 5(A)]. The octanoyl chain (C8) of the inhibitor is accommodated in an extended conformation in the highly hydrophobic tunnel [Fig. 5(C)], while the ring moiety of the inhibitor, which mimics the lactone ring, is partly disordered. A water molecule bridges an interaction between the carbonyl oxygen of the ring and the side chain oxygen of Ser148. The nitrogen and oxygen atoms of the ring form hydrogen bonds with the main chain of Arg104 and Phe105. It should be noted that the TofI binding pocket appears to be highly specific to C8 acyl-chain, because J8-C6 (containing a hexanoyl chain) is not an inhibitor.⁸⁷

The three structurally characterized LuxI members (EsaI, LasI, and TofI) produce HSLs of varying

acyl chain length, and a comparison of their acyl chain binding pockets affords some insights into the determinants of specificity. A superposition of the EsaI structure onto the TofI-C8-HSL-J8-C8 ternary structure shows that the acyl chain-binding tunnel is significantly shorter in EsaI (which generates a C6 product) [Fig. 5(D)]. Although residues that line this tunnel occupy similar positions in both TofI and EsaI, two residues located at the end of the tunnel show significantly different spatial arrangement. The side chain of Thr140 and Leu176 in EsaI points into the tunnel and blocks the potential path of the acyl chain. Their counterparts in TofI are Gly143, which lacks a side chain, and Ile179 that is oriented in a different conformation away from the tunnel. The location of these two residues within EsaI may explain its substrate preference for a C6 acyl chain substrate.

A superposition of the LasI structure with the TofI ternary complex structure reveals that the acyl chain-binding tunnel is nearly completely occluded in the former [Fig. 5(E)]. In the LasI structure, Phe105 juts into the tunnel at a position that would correspond to the location of C3 of the acyl chain. Interestingly, Phe105 occupies two different conformations in the LasI structure, but neither conformation would allow for accommodation of the C12 acyl chain of the LasI substrate. While it is tempting to speculate that the larger acyl chain of the LasI

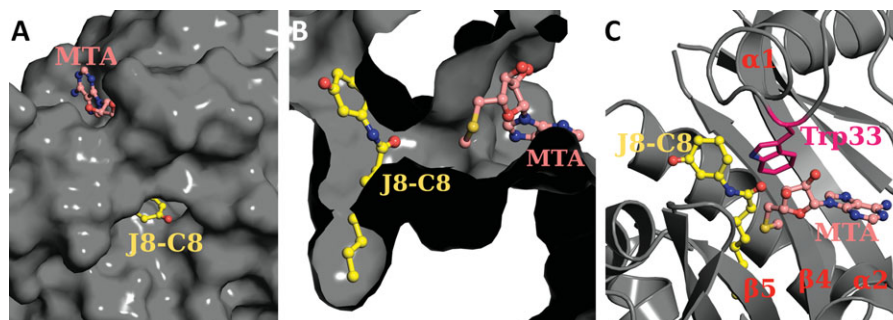


Figure 6. Binding of 5'-methylthioadenosine (MTA) to TofI. (A) Top view of the binding sites of MTA and J8-C8. (B) The tunnel between J8-C8 and MTA. (C) Close-up view of the MTA binding site.

substrate must be bound in a different (hitherto unidentified) binding pocket, as suggested by modeling studies on EsaI and LasI,^{86,89} such theories cannot be accepted in the absence of any experimental data.

Probable binding modes for ACP and SAM to LuxI-type AHL synthases

As noted above, in the TofI ternary complex structure, the ring moiety of J8-C8 is partially disordered but is modeled to be situated at the exposed inlet of the acyl chain tunnel, which is surrounded by the C-termini of helix $\alpha 1$ and strands $\beta 4$ -5, and the N-termini of helices $\alpha 4$ -5, and strand $\beta 7$ [Fig. 5(A)]. Therefore, residues in this vicinity are presumably involved in the interaction with both the phosphopantetheine group and ACP protein tether. This is in agreement with the observation in the modeling studies of both EsaI and LasI.^{85,86}

A docking model of LasI in complex with 3-oxo-C12-acyl-phosphopantetheine suggests that residues from strands $\beta 5$ and $\beta 7$, and helix $\alpha 5$ may interact with the phosphopantetheine group and ACP protein.⁸⁶ It has been suggested that ACP proteins interact with partner proteins largely through electrostatics and the binding interface on the ACP is rich in anionic residues.^{90,91} Consistent with these findings, a group of basic residues are found in the predicted regions of LasI, including Lys150, Arg154, Arg161, His165, Lys167, and Arg172.⁸⁶ These residues form a highly positively charged surface that would interact with the negative surface of ACP. Most of these residues are conserved in EsaI and TofI [Fig. 4(A)], and their importance is further supported by mutagenesis studies. Single or double mutations at these residues resulted in a deficiency in AHL synthesis, presumably as a result of the inability to engage ACP-bound acyl substrates.⁸⁶

TofI binds to the product of SAM turnover (MTA) in a solvent exposed site that is approximately 8 Å away from the J8-C8 binding pocket⁸⁷ (Fig. 6). The binding sites for the two ligands are on the opposite sides of the central β -sheet but appear to be connected through the V-shaped cleft between strands $\beta 4$ and $\beta 5$. An apparent channel is observed

between J8-C8 and MTA [Fig. 6(B)], and this channel presumably accommodates the majority of the methionine moiety of the SAM substrate. The methylthiol group of the MTA molecule inserts into the binding pocket and points toward J8-C8, while the adenosine group highly exposed on the protein surface. Several β -strands surround the MTA binding region on one side, while the other side is composed of two short helices $\alpha 2$ -3 and a long loop connecting $\alpha 1$ and $\beta 1$, which is disordered in the apo TofI structure but becomes ordered upon ligand binding. Trp33 on this loop provides the important stacking interaction to MTA 5'-methylthiolribose ring [Fig. 6(C)], and mutational analysis confirms that this stacking interaction is critical for enzyme activity.⁸⁷ Notably, the equivalent loop in LasI structure would coincide with the position of the MTA molecule, implying that a large movement of the loop is required to accommodate the SAM substrate.

Putative reaction mechanism of LuxI-type AHL synthases

While the reaction mechanism of LuxI-type AHL synthases is not yet fully understood, a mechanism has been proposed for RhII⁹² based on analogy to the acetyltransferase mechanism of GCN5 superfamily enzymes.^{93,94} In this mechanism, the α -amine of SAM is activated by proton abstraction by a hydroxide ion, facilitating nucleophilic attack on the carbonyl C1 atom of the substrate acyl chain (Fig. 3). A suitably poised solvent molecule is found in the vicinity of a conserved glutamic residue in the structures of EsaI (Glu97), LasI (Glu101), and TofI (Glu101) [Fig. 4(A)], and mutation at this residue abolish enzyme activity of several AHL synthases, including RhII,⁷⁰ EsaI,⁸⁵ and TofI,⁸⁷ consistent with the role of this glutamic acid as a general base.

Lactonization of the ring is proposed to follow the acylation step in this mechanism.⁹² Enzyme inhibition studies on RhII showed that butyryl-SAM can act as a substrate and also as an inhibitor of AHL synthesis.⁷⁸ These results suggested that acyl-SAM may be a reaction intermediate and that acylation precedes lactonization. The formation of acyl-

SAM as an intermediate was further confirmed by transient-state kinetic studies.⁹⁵ Within the acyl-SAM intermediate, the cyclization of the methionine moiety appears to proceed via a direct nucleophilic attack on C γ by the carboxylate oxygen, as shown by deuterium incorporation studies on RhlI.⁹²

The order of substrate binding to AHL synthases is still under debate. Studies on RhlI suggested that AHL synthesis occurs through a bi-ter sequential ordered reaction,⁷⁸ with SAM binding before acyl-ACP. However, it was reported that EsaI can form complexes with both acyl-ACP and holo-ACP (phosphopantetheine group linked to ACP protein) *in vitro* without SAM,⁸⁵ suggesting that acyl-ACP binds first. It should also be noted that, in the TofI ternary complex structure, the binding sites for the acyl-substrate and SAM are well separated and they appear to pose little impact on each other.⁸⁷

LuxR-Type AHL Receptors

AHL signal molecules are recognized by LuxR-type receptors, which constitute a class of transcription factors that possess an amino-terminal AHL-binding domain and a carboxy-terminal DNA-binding domain.^{96,97} Most characterized LuxR-type receptors are transcriptional activators that are positively regulated by cognate AHL molecules. For example, the binding of 3-oxo-C6-HSL to LuxR protein from *V. fischeri* triggers receptor binding to the promoter of target genes to activate gene expression. The expression of the AHL synthase LuxI is also upregulated by LuxR, resulting in a positive feedback loop¹ (Fig. 1). LuxR-type AHL receptors that function as transcription repressors have also been reported, with the best studied example being EsaR from *P. stewartii*.⁶⁴ Binding of the 3-oxo-C6-HSL AHL signaling molecule to the EsaR receptor negatively regulates gene repression. EsaR binds to the promoter of target genes in the absence of 3-oxo-C6-HSL and represses the expression of these genes. Upon binding of the AHL signal molecule, EsaR dissociates from its DNA targets and the gene expression is relieved.⁶⁴

The understanding of the mechanism of signal perception by the LuxR-type AHL receptors has been largely based on structural studies. The crystal structures of five LuxR-type AHL receptors have been reported, including TraR_{At} from *Agrobacterium tumefaciens*,^{98,99} TraR_{NGR} from *Rhizobium* sp. NGR234,¹⁰⁰ LasR,¹⁰¹ and QscR¹⁰² from *P. aeruginosa*, and CviR from *Chromobacterium violaceum*.¹⁰³ The NMR structure of another receptor of this type has also been reported, SdiA from *Escherichia coli*.¹⁰⁴ Overproduction of soluble recombinant LuxR-type proteins for biochemical and structural characterization required the addition of the cognate AHL molecules to the bacterial media.¹⁰⁵

Overall structure of LuxR-type AHL receptors

Among the structurally characterized LuxR-type AHL receptors, four proteins have been crystallized as full length constructs, including TraR_{At},^{98,99} TraR_{NGR},¹⁰⁰ QscR,¹⁰² and CviR,¹⁰³ while only the AHL-binding domain structures have been reported for LasR¹⁰¹ and SdiA.¹⁰⁴ The full-length structures show that LuxR proteins are homodimers composed of two domains, a large N-terminal domain (~170 residues) and a small C-terminal domain (~65 residues) [Fig. 7(A)], which are connected through a highly flexible linker loop (~10 residues). The overall fold of each domain is highly conserved, with an RMSD of 2 to 4 Å over ~150 C α atoms for the N-terminal domain, and 1.2 to 1.7 Å over ~60 C α atoms for the C-terminal domain.⁸⁸ However, the relative orientation of the two domains is highly dynamic, as demonstrated by the fact that the full-length structures reported so far all show different overall conformations. Even within the dimeric structure of an individual LuxR protein, the two monomers may display different conformations, as illustrated by the structures of TraR_{At} [Fig. 7(A)]^{98,99} and TraR_{NGR}.¹⁰⁰

The N-terminal domain of LuxR proteins shows an overall fold that is similar to a GAF/PAS domain, which has been identified in numerous multidomain proteins involved in signal transduction processes.¹⁰⁶ It is composed of a central β -sheet sandwiched by two groups of α -helices [Fig. 7(A)]. The β -sheet typically has four or five strands, and there are usually five or six major α -helices, with α 1- α 2- α 5 on the convex side of the β -sheet and α 3- α 4 on the concave side. The C-terminal domain is folded into a four-helical bundle structure with a helix-turn-helix motif (α 7- α 8) [Fig. 7(A)], a DNA-binding motif found in numerous transcription factors.¹⁰⁷ LuxR proteins are classified among the large FixJ-NarL superfamily as the sequence of the C-terminal domains shows homology to the C-terminus of other members in this superfamily.^{108,109} In contrast to most members of this superfamily, whose N-termini undergo phosphorylation as a part of the typical two-component signal transduction process, the N-terminal domain of LuxR is not phosphorylated but rather involved in binding to the AHL ligands.

As noted, LuxR proteins have been shown to homodimerize in the presence of cognate signal^{110,111} and the extent of dimerization is dependent on the concentration of the ligand.¹¹² Consequently, available structures of LuxR proteins, all of which have a bound AHL molecule or inhibitor, are homodimeric, with the exception of the NMR structure of SdiA. In all these dimer structures the dimerization contacts are mostly formed by hydrophobic interactions between the N-terminal domains, and all involve helices α 1 and α 5, which are located on the convex side of the central β -sheet. However, there are apparent variations amongst the dimer interface.

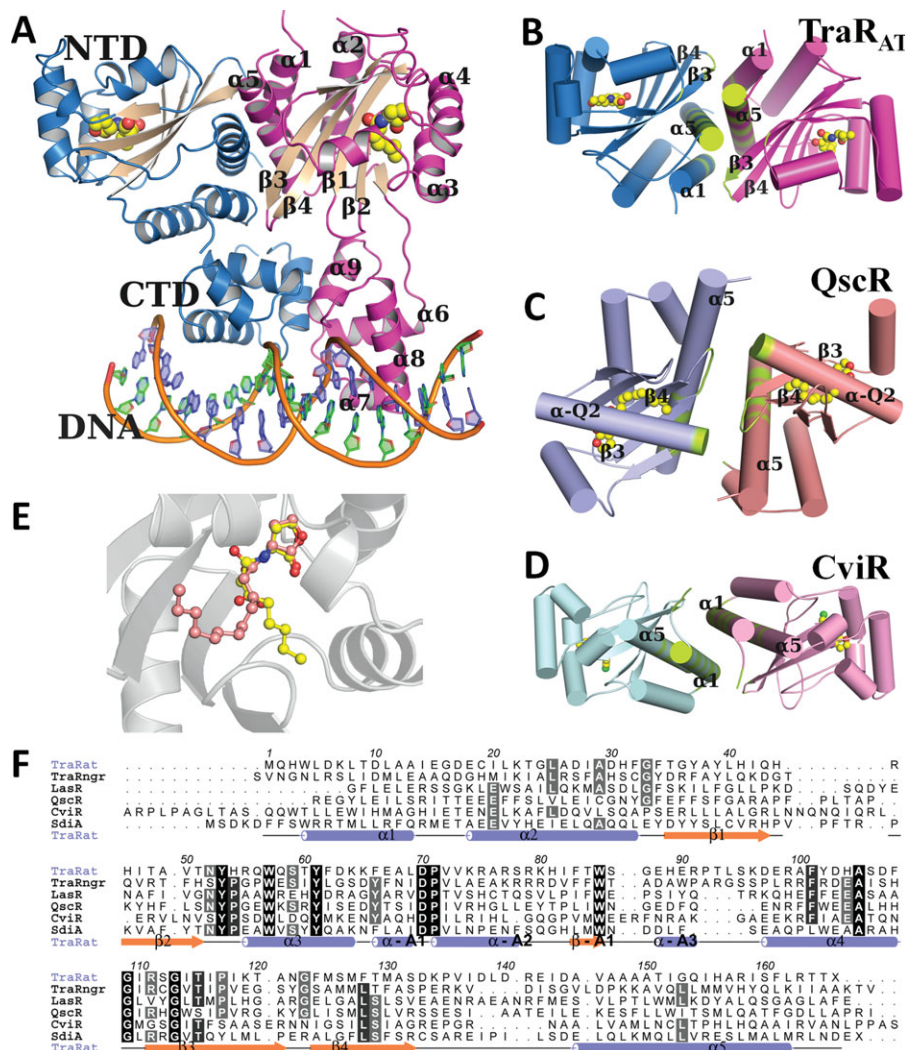


Figure 7. Structures LuxR-type AHL receptors. (A) Overall structure of TraR_{AT}-AHL-DNA ternary complex. The AHL ligand (3-oxo-C8-HSL) is shown as yellow spheres. NTD: N-terminal domain; CTD: the C-terminal domain. (B–D) Dimerization of the NTD of TraR_{AT}, QscR, and CviR. The residues on each monomer that are within 5 Å of the opposite monomer are shown in green. The bound ligands are shown as yellow spheres. (E) Comparison between the AHL-binding modes of TraR_{AT} and QscR; 3-oxo-C8-HSL bound to TraR_{AT} (gray) is shown in yellow and 3-oxo-C12-HSL bound to QscR (not shown) is shown in pink. (F) Structure-based sequence alignment of the NTD of LuxR receptors. Secondary structure of TraR_{AT} is shown below the sequences.

In the structures of both TraR_{AT}^{98,99} and TraR_{NGR}¹⁰⁰ symmetric interactions are formed between helices $\alpha 5$ of the two monomers and also between helix $\alpha 1$ of one monomer and the loop connecting strands $\beta 3$ and $\beta 4$ in the opposite monomer [Fig. 7(B)]. Helices $\alpha 1$ and $\alpha 5$ of one monomer are nearly parallel to those in the opposite monomer. The dimer interfaces of QscR¹⁰² and LasR¹⁰¹ are similar with each other but distinct to that of TraR_{AT} and TraR_{NGR}. Dimer formation is mediated by interactions between the $\alpha 5$ helices of each monomer and between helix α -Q2 of one monomer and the loop connecting strands $\beta 3$ and $\beta 4$ in the opposite monomer. The relative orientation of the two monomers in QscR dimer is in striking contrast to that in TraR, and the interacting helices $\alpha 5$ in QscR are vertical to each other [Fig. 7(C)]. Distinct to

these two types of dimer interfaces, the dimerization interactions in CviR¹⁰³ are mainly contributed by symmetrical contacts between helix $\alpha 1$ of one monomer and both helices $\alpha 1$ and $\alpha 5$ of the opposite monomer. In this conformation, helices $\alpha 1$ and $\alpha 5$ of one monomer are stacked to those of the opposite monomer, forming a four-helical bundle arrangement [Fig. 7(D)].

Although in the full-length homodimeric structures of TraR_{AT}^{98,99} and QscR¹⁰² the C-terminal domains also interact with each other, such interactions do not appear to be required for dimerization. This is evidenced by the fact that TraR_{AT} can form a heterodimer with TrIR, which is highly homologous to the N-terminal domain of TraR_{AT} but lacks the C-terminal domain.¹¹³ In addition, the N-terminal domain of LasR is sufficient for homodimerization and

has the same dimer interface as the full-length QscR dimer.¹⁰¹

AHL recognition by LuxR-type receptors

All of the LuxR protein structures were reported in complex form with their cognate AHL molecules. The AHL molecule is deeply buried in a pocket enclosed by helices $\alpha 3$ - $\alpha 4$ and the concave side of the central β -sheet [Figs. 6(E) and 7(A)]. The homoserine lactone and the acyl chain of the AHL molecule are accommodated by extensive hydrophilic and hydrophobic interactions. The polar residues that coordinate the homoserine lactone moiety include Trp57, Trp59, and Trp60 (TraR_{AT} numbering), which are all completely conserved amongst LuxR proteins [Fig. 7(F)]. The residues that provide hydrophobic and van der Waals interactions to the AHL ligand are less conserved, and this is suggested to account for the diversity of acyl chains in different AHL molecules, as shown by mutagenesis studies on QscR.¹⁰² The extensive protein-ligand interactions imply that the AHL molecule might be responsible for stabilizing the overall structure of the ligand-binding domain, which might explain why heterologous expression of soluble LuxR proteins is facilitated by the addition of the AHL signal during protein production.

A comparison of the binding modes of different AHL molecules with their cognate receptors revealed that, although the homoserine lactone moiety is largely fixed in an orientation defined by the three conserved polar residues, the acyl chains of these AHL molecules occupy two different cavities. The shorter AHL molecules, including 3-oxo-C8-HSL, 3-C8-HSL, and C6-HSL that are bound with TraR_{AT}, SdiA, and CviR, respectively, adopt an extended conformation in the binding pocket and point toward solvent [Fig. 7(E)]. In contrast, 3-oxo-C12-HSL bound by LasR and QscR show a curled conformation in which the long acyl chain points toward the interior of the binding pocket and exploits the inner surface of the pocket along the concave side of the central β -sheet [Fig. 7(E)]. Therefore, it appears that different LuxR proteins accommodate diverse AHL molecules using a strategy that combines both amino acid variation and flexibility in the binding pocket.

DNA binding by LuxR-type receptors

LuxR proteins bind to DNA targets through the C-terminal domain.^{96,114} The C-terminal domain of *V. fischeri* LuxR was capable of acting as a transcription activator when overexpressed in *E. coli*.⁹⁶ The DNA targets of LuxR proteins, termed *lux* boxes, are typically inverted repeats that lie in the promoter region of the target genes.^{105,115–118} For example, in *A. tumefaciens* the target promoters of TraR_{AT} contain one or multiple copies of an 18-bp palindromic

site called *tra* box.^{117,118} For LuxR proteins that act as transcription activators, when binding to the *lux* box (−42.5 position upstream of *luxI*), they interact with the C-terminal domain¹¹⁹ and the sigma subunit¹²⁰ of RNA polymerase (RNAP). For repressors in the LuxR family, for example, EsaR, they bind to the *lux* box that is near the −10 position and thus interrupt promoter binding by RNAP.^{64,121}

Two different groups have reported the crystal structures of TraR_{AT} in complex with its cognate AHL ligand and the *tra* box.^{98,99} The TraR_{AT} ternary structure shows that the *tra* box is bound to the C-terminal domains of a TraR dimer, with each domain binding to the major groove of one 9-bp half-site [Fig. 7(A)]. Protein-DNA contacts results in an approximate 30 degrees bend in the DNA duplex. The C-terminal domain of TraR_{AT} consists of four helices, $\alpha 6$ - $\alpha 7$ - $\alpha 8$ - $\alpha 9$, and the typical helix-turn-helix motif is formed by helices $\alpha 7$ and $\alpha 8$, which are known as the “scaffold” and “recognition” helices, respectively. The two helices are stabilized by hydrophobic interactions within the four-helical bundle structure, as well as a conserved salt bridge between Glu178 ($\alpha 6$) and Arg215 ($\alpha 8$) [Fig. 8(A)].^{98,99}

The interactions between each monomer and the corresponding half-sites are symmetrical. The recognition helix $\alpha 8$ lies in the major groove and is oriented perpendicularly to the DNA axis of the half-site. Base-specific interactions are attributed to residues Arg206 and Arg210, with the former interacting with G13 of strand E and G5-C6 of strand F, and the latter interacting with C14 of strand E and T4 of strand F [Fig. 8(B)]. The importance of these nucleotides for TraR binding were confirmed by *in vitro* and *in vivo* assays.¹²² Interactions with DNA backbone sugar and phosphate groups involve both polar and nonpolar residues in helices $\alpha 7$, $\alpha 8$, and $\alpha 9$. The central six nucleotides of the *tra* box, although do not form direct contact with TraR, are critical for high-affinity binding, presumably by facilitating a DNA bend upon TraR binding.¹²² On the other hand, alterations of the outermost nucleotides in the *tra* box had only small impact in TraR binding but prevented transcription, which is probably due to destruction of the RNAP binding site.¹²²

Notably, the full length structures of the two TraR monomers adopt two different conformations, with one in an open conformation and the other one in a closed conformation [Fig. 7(A)]. This asymmetry results in an optimal interaction between the two C-terminal domains and the two half-sites. Interestingly, the full length dimer structures of TraR_{NGR}¹⁰⁰ and CviR¹⁰³ display dramatically different conformations compared to TraR_{AT}. In both structures the C-terminal domains are separated by a large distance that would preclude binding to the cognate DNA target. This unproductive conformation is formed by the binding of an antiactivator protein,

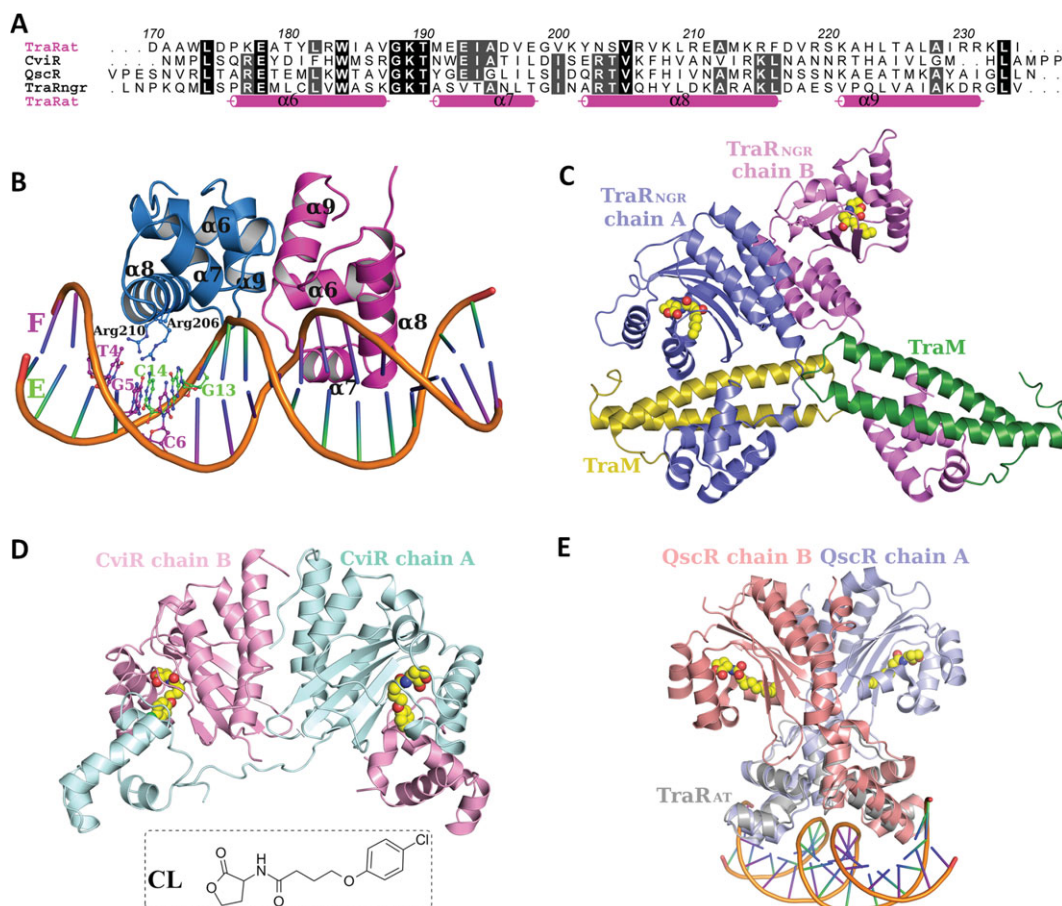


Figure 8. C-terminal domain of LuxR-type AHL receptors. (A) Structure-based sequence alignment of the C-terminal domains of four LuxR proteins. Secondary structure based on TraR_{AT} is shown below the sequences. (B) The C-terminal domains of TraR_{AT} dimer binding to the *tra* box. Arg206 and Arg210 of one monomer and their interacting bases are shown as ball-stick model. Strands E and F of the DNA are indicated. (C) TraR_{NGR} binding to antiactivator TraM. (D) CviR binding to a chlorolactone compound (CL, yellow). (E) QscR dimer superimposed with TraR_{AT}-AHL-DNA complex. The C-terminal domains of TraR_{AT} are shown in gray. The N-terminal domains of TraR_{AT} are not shown.

TraM, to TraR_{NGR}¹⁰⁰ [Fig. 8(C)] or binding of a small molecule antagonist to CviR¹⁰³ [Fig. 8(D)]. In contrast, the C-terminal domains in the full-length QscR form a dimer that is similar to that in the TraR_{AT} structure, with an RMSD of 1.5 Å over 118 C_α atoms¹⁰² [Fig. 8(E)]. It was suggested that the QscR dimer structure, although in a different overall

conformation compared with TraR_{AT}, may be competent for DNA-binding.¹⁰²

Binding of inhibitors or activators by LuxR-type receptors

High throughput screening of synthetic and natural product libraries has identified several small

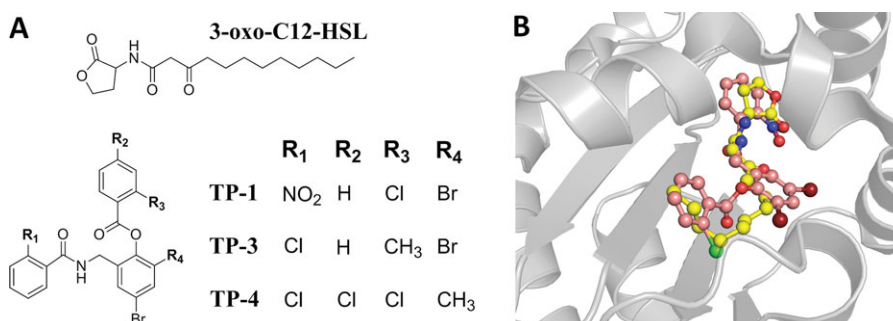


Figure 9. Triphenyl compounds binding to LasR. (A) Chemical structures of the cognate ligand (3-oxo-C12-HSL) of LasR and triphenyl (TP) compounds TP-1, TP-2, and TP-3. (B) Comparison between the binding modes of 3-oxo-C12-HSL (yellow) and TP-1 (pink) to LasR.

molecule inhibitors of QS.^{123–127} The recent investigations on CviR protein from *C. violaceum* strains ATCC 31532 (CviR) and ATCC 12472 (CviR') explain the rationale behind LuxR-type receptor antagonism by a series of small molecules.¹⁰³ *C. violaceum* strain ATCC 31532 produces and responds to C6-HSL. The recognition of this native signal molecule by its receptor CviR is strongly antagonized by C8-HSL, C10-HSL, and a chlorolactone compound (CL).¹⁰³ Crystal structure of CviR-CL complex showed that the antagonist induces the receptor dimer into a "crossed-domain" conformation, in which the DNA-binding domain of each extended monomer lies underneath the ligand-binding domain of the opposite monomer¹⁰³ [Fig. 8(D)]. This conformation separates the two DNA-binding domains and thus precludes binding to the DNA target. In this closed dimer conformation a large portion of the surface area of the two monomers is buried at their contact interface.

A similar closed dimer conformation was also observed in the structure of the CviR homolog (CviR') from *C. violaceum* strain ATCC 12472.¹⁰³ In addition to its cognate ligand (3-hydroxy-C10-HSL), CviR' also responds to C10-HSL, while C6-HSL acts as a partial antagonist. The crystal structure of CviR'-C6-HSL complex shows a closed dimer conformation that is not competent for DNA binding.¹⁰³

A renewed interest in the pharmacological studies of QS has been spurred by the identification of structurally novel (i.e. distinct from cognate ligands) small molecules that can interact with LuxR type receptor. A high-throughput screen of a library of 200,000 compounds identified several small molecules, based on a triphenyl (TP) scaffold, which can activate LasR but are structurally unrelated to AHL molecules [Fig. 9(A)].¹²⁶ The co-crystal structures of the ligand-binding domain of LasR in complex with several of these triphenyl scaffold bearing molecules showed that these compounds bind LasR in the AHL-binding pocket and interact with the protein in a similar manner to the cognate AHL molecule 3-oxo-C12-HSL [Fig. 9(B)].¹²⁸ These studies provide a conclusive case study that pharmaceutical intervention of LuxR signaling can be achieved through small molecules, paving the way for future drug-discovery efforts that focus on QS.

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